Report to NRSP-6 Technical Committee
J. Creighton Miller, Jr.
June 29-30, 2004

Potato Research Programs and Use of NRSP-6 Stock in the Southern Region

There are three states in the Southern Region with on-going active potato improvement and/or research programs utilizing NRSP-6 stock: North Carolina, Texas, and Virginia. Several other states periodically conduct potato research utilizing NRSP-6 stock.

2003 SOUTHERN REGIONAL ORDERS

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Units: 30 Pilot test for jelly end screening in 2004.

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Units: 6 Research with SDR/FDR populations.

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Units: 6 Herbarium specimens of Texas collections for the SRSC herbarium.

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Units: 12 Potato varieties for microtuberization studies:

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Units: 6 Experiments to analyze changes in gene expression with drought stress.

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Units: 75 tuberization testing:
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*** = INFORMATION NOT PROVIDED BY COOPERATOR

Texas – J. Creighton Miller, Jr. The Texas Potato Variety Development Program continues to strive for the development and identification of improved early maturing russet, colored flesh, chip, and red varieties adapted to Texas growing conditions in order to enhance the competitiveness of the Texas potato industry. In 2003, 119,851 first-year seedlings representing 670 families were grown and 238 original selections were made. We cooperate with the North Dakota, USDA/ARS Aberdeen, ID, USDA/ARS Madison, WI, Oregon, Minnesota, and Colorado breeding programs through exchange of first-year seedling tubers and/or advanced selections. We continue to participate in the Western Regional Trials (russet, red/specialty and chipping) and the Southwestern Regional Trials (russet, red, and specialty). Advanced selections near release include NDTX4271-5R, NDTX4304-1R, and NDTX4930-5W. TX1523-1Ru/Y was released in 2003 and is sold under the name Sierra Gold™. Virtually all of the russet potatoes grown in Texas in 2003 were to the improved Texas Russet Norkotah strains. We expanded our capabilities to evaluate processing /chipping selections and our effort to develop improved red and colored flesh varieties. When this program was initiated in 1973, the average yield of the summer crop in Texas was about 200 CWT. In 2002 the average summer crop yield in Texas was reported to be 400 CWT, the highest in the nation among 15 states with summer crop production.

Total antioxidant activity and total carotenoid levels were evaluated for more than 100 common potato *(Solanum tuberosum,* L.) cultivars grown in the United States, advanced breeding lines from several Western U.S. breeding programs, and 47 related, tuber-bearing species. An initial assessment of variability for antioxidant activity provided baseline information to be used for potential potato promotion and for the development of new cultivars with greater human health benefits. The *pinnantisectum* accessions consistently ranked among the highest in antioxidant

activity. However, topping the list was a *cardiophyllum* accession (341235) followed by an accession of *commersonii* (320266). The highest of the *jamesii* samples was accession 603056. This sample was 4 fold higher than the lowest *jamesii* sample. A broad screen was also conducted for carotenoids. Again, *pinnantisectum* consistently ranked among the highest along with *commersonii* accession 320266. Statistical analysis revealed a correlation between carotenoid and phenolic results.

This work was conducted as part of a cooperative research agreement with Dr. John Bamberg, and involved both broad and fine screening for carotenoid and phenolic compounds. Wide variability in antioxidant levels provided evidence of genetic control of this trait, indicating that it could be possible to breed for enhanced levels of antioxidant compounds in potato. Accessions, cultivars, and advanced breeding lines identified in the broad screen as having high antioxidant activity and high total carotenoid levels, were fine screened via HPLC to determine specific phenolic and carotenoid compounds present in potato. The objective of the study was to identify parents for use in the Texas breeding program in order to develop potato cultivars containing increased levels of antioxidant compounds.

In the broad screen for total antioxidant activity, the 47 related, tuber-bearing species showed a wider range of variability than the named cultivars and breeding lines. Based on the DPPH assay, antioxidant activity ranged from 103-648 uM trolox equivalents in the named cultivars and advanced breeding lines, while that of the wild species was 42-892. HPLC analysis revealed that the phenolic content of the species, and their cultivated counterparts, was primarily composed of caffeic and chlorogenic acids. Other phenolics identified were p-coumaric acid, rutin hydrate, vanillic acid, epicatechin, t-cinnamic acid, gallic acid, and salicylic acid. The highest phenolic content discovered in the accessions was five-fold higher than the highest of the cultivated genotypes. Carotenoid analysis revealed lutein in the accessions, but the yellow-flesh breeding lines were much higher in carotenoids.

North Carolina - G. Craig Yencho The North Carolina program focuses on two areas: the development of new potato germplasm and varieties through collaborative early-generation breeding and selection projects with the USDA-ARS, Cornell University and the University of Maine; and the evaluation of preliminary and advanced breeding clones for adaptation to NC from a wide range of potato breeding programs in the US and Canada. Even though the NCSU program does not utilize the NRSP-6 collection as extensively as the larger breeding programs located in the north, we realized that many of our northern collaborators do, thus our industry benefits greatly from these indirect interactions. In 2003, we planted 8,364 single-hill plots and made 236 selections. The TPS was derived from USDA-ARS and NCSU crosses. We also planted 202 four-hill plots from the University of Maine and the USDA, 167 six-hill plots from last year's single-hill selections in NC, 48 12-hill plots from the University of Maine and Cornell University, and 63 20-hill plots from the USDA and the University of Maine. In addition, a total of 197 preliminary and advanced clones were evaluated in replicated yield trials either on-farm, or at the Tidewater Research Station (NCDA&CS)/Vernon G. James Research and Extension Center, (NCSU) in Plymouth, NC.

In 2003, the USDA clones with the most potential as chippers were: Harley Blackwell, and B0766-3. In all cases, yields were good and chip scores were 3 or better. The clone Harley Blackwell, formerly known as B0564-8, was released by the USDA-ARS during 2003. It is an attractive, round-white potato with a medium net skin and an average specific gravity in NC of 1.073 compared to 1.076 for Atlantic. Harley Blackwell is primarily intended for chipping, but its shallow eyes, relatively attractive appearance, and lower gravity compared to Atlantic may also make it suitable for table-stock purposes. In NC, it has produced marketable yields 108% of Atlantic. It does not suffer from internal heat necrosis (IHN), a common problem of Atlantic in the mid-Atlantic and SE US.

The table-stock clones from the USDA with the most potential were: B1806-8 a round, yellow-fleshed potato; B1758-4 a red-skinned, white-fleshed clone; and B1816-5 a purple-skinned, yellow-fleshed clone. In terms of internal defects B1806-8 and B1758-4 have expressed hollow heart, brown center and some soft rot but overall incidence has been less than 10%. B1816-5 has been consistently free of major defects, and we believe that this clone has good potential as a specialty-type potato. We will continue testing it with the NCSU Specialty Crops Program and on-farm in 2004.

Clones from the University of Maine showing the most promise were: AF1424-7 and AF1569-2. In most trials the marketable yields for these were good, there were few internal defects, and chip scores for were either 2 or 3. From Cornell University NY112 (recently released as Marcy), NY126, and NY129 performed well. Marcy (NY112) is an especially attractive, netted, chip-stock potato with good yield. However, it has suffered from susceptibility to IHN making it potentially unsuitable for NC. NY126 and NY129 have promise as table-stock varieties and we will continue intensive evaluations of these clones. NY126 is an attractive, round to oblong, pale yellow-fleshed potato with yields close to Atlantic. NY129 is a round, red-skinned, white-fleshed potato with few internal defects and yields similar to Chieftain. Two clones from Michigan of note are: MSI005-20Y and Michigan Purple. MSI005-20Y is an attractive light yellow flesh, white skin potato with few internal defects though its yields were off. Michigan Purple is a high yielding purple skin, white flesh potato.

Ongoing germplasm enhancement efforts currently underway at NCSU include the following.

1. We are continuing to evaluate a set of 4x tbr-chc leptine-producing clones for resistance to CPB in the field and greenhouse (the initial crosses for this work were made in 2000 and 2001). A graduate student (MS), Mr. Trevor Chlanda, has begun a study focused on identifying cAFLP markers associated with leptine production and CPB resistance in three F2 progenies derived from these materials.

2. With Dr. Merideth Bonierbale of the International Potato Center (CIP) Lima, Peru, we completed at USDA-FAS project focused on improving the germplasm base of heat tolerant potatoes in the US. To do this, we introduced to the US four parents of CIP's "Lowland Tropics Population." This population was developed for use in the hot, humid climates of the lowland tropics. The parental materials have been transferred to the

NRSP-6 project. In 2001/2002, we commenced the crossing and parental evaluation work of this project. The parental clones (4) were crossed to US material during 2002/2003, generating over 12,000 seed from 16 families, which are currently under evaluation.

3. With Dr. Kathy Haynes, USDA-ARS BARC, we are conducting a multiyear genotype x environment internal heat necrosis (IHN) experiment. This research is being conducted in collaboration with the USDA-ARS BARC, Rutgers University, and VA Tech. The goal of this project is twofold. First, we wish to develop new sources of high specific gravity clones free of IHN that can then be used to develop new chipping cultivars to replace Atlantic, a clone that is highly susceptible to IHN. Second, we want to better understand the physiological basis of resistance/susceptibility to IHN. In 2003, with support from the ARS, we conducted two replicated field trials (35 clones total) to evaluate the abovementioned materials for resistance/susceptibility to IHN. In addition, DNA was extracted from 17 of these clones and bulked segregant analysis (BSA) was carried out on the 5 most IHN-resistant and 5 most IHN-susceptible clones, using the amplified fragment length polymorphism (AFLP) technique with fluorescently labeled EcoRI primers. A total of 192 primer combinations were run on each bulk to identify putative DNA polymorphisms. These were verified on the individuals of the bulks. Using the verified combinations, reactions were run again using ^{32}P-labeled primers, and 35 polymorphic fragments were excised, cloned and sequenced. Database searches using **BLAST** (Basic Local Alignment Search Tool) are currently underway. Preliminary results include significant matches with several retrotransposons (7 fragments, including 5 only found in IHN-susceptible individuals), R-gene clusters from S. tuberosum (3 fragments), ESTs (Expressed Sequence Tags) from tomato and S. tuberosum cDNA libraries (7 fragments), and two known proteins: a calcium-dependent kinase, and an anthocyanidin-3-glucoside rhamnosyltransferase, both from Arabidopsis thaliana.

4. In 2002, we began a project with Dr. John Bamberg, in which 75 accessions representing 20 different potato species were evaluated for their ability to form tubers in the mountains of NC. This project was initiated because it has been observed that a number of accessions do not produce adequate numbers of tubers at the Sturgeon Bay, WI site given the short growing season. During 2002/2003, we planted 20 hills of each accession and only five of the 75 families did not produce tubers, while only 26 produced < 100 grams of tubers, but only after a prolonged season and virus pressure was significant. Based on this, it does not look like western NC is a suitable location for the production of theses materials.

Virginia – Richard E. Veilleux. DNA sequences that flank the *Tst1* retrotransposon sites in monoploid clones of *Solanum phureja* were amplified using an outward directed primer anchored in the retrotransposon and a random AFLP primer. The sequences were converted to probes and used in Southern blots of restricted DNA extracted from a diverse group of monoploids as well as other solanaceous genera. Most of the probes were polymorphic in *S. phureja,* yielding few to several bands. All of the probes that were isolated from S. phureja hybridized to cultivars of S. tuberosum. Some (8 of 11) also hybridized to DNA extracted from tomato; however none hybridized to DNA of *Arabidopsis*.

Bacterization of sibling monoploids extracted from a single heterozygous selection of S. phureja with *Burkholderia phytofirmans* strain PsJN-D revealed considerable variation among the monoploids for the ability to enter into a positive association with the endophyte. Positive controls exhibited increased fresh shoot weight, reduced root length and increased root weight in response to bacterization. Some monoploids behaved similarly to the controls whereas others displayed no response to inoculation with the endophyte.

Diploid potato clone SMP-C, a hybrid between *S. phureja* BARD 1-3 and the haploid inducing pollinator, IVP 101, was transformed with the GFP (green fluorescent protein) gene modified for expression in higher plants. One of the transformed calluses regenerated diploid plants and two others regenerated tetraploid plants. Segregation ratios for tetraploid T1 seedlings fit models for single duplex insertions (35 transgenic: 1 non) or double simplex insertions (15 transgenic: 1 non). Diploid T1 seedlings segregated for deleterious traits: dwarfed size and curled leaves, as well as the GFP transgene. There was substantial GFP silencing evidenced by the loss of expression in plants that had originally been selected for high GFP. However, six selections were found to be
free of deleterious traits, consistently high expressers of GFP, and producers of stainable pollen with less 2n than IVP 101.

Transgenic PVY coat protein mediated resistance was examined in seven primary transformants, three of *S. tuberosum* cv. Atlantic, two of diplandriod O5-10 and two of diplandroid APM-2. One O5-10 diplandroid exhibited high levels of resistance with no visual symptoms after inoculation with the virus and no translocation of virus to uninoculated leaves. The resistance was comparable to control resistant cvs. Bison and Corinne (obtained from the NRSP-6 germplasm repository).

Publications:

Palumbo, R. 2004. The potential for green fluorescent protein as a screening tool in the production of haploid potato plants. M.S. thesis, Virginia Polytechnic Institute & State University, Blacksburg

Lightbourn G* and Veilleux, R, 2003. Retrotransposon based markers to characterize somatic hybrids and assess variation induced by protoplast fusion of monoploid potato. Acta Hort. 619:35-43